

# Susceptibility of Last Instar Red Flour Beetles and Confused Flour Beetles (Coleoptera: Tenebrionidae) to Hydroprene

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**ABSTRACT** Last instar larvae of the red flour beetle, *Tribolium castaneum* (Herbst), and the confused flour beetle, *Tribolium confusum* Jacquelin du Val, were either exposed for 8–144 h on concrete treated with  $1.9 \times 10^{-3}$  mg(AI)/per cm<sup>2</sup> hydroprene, or continually exposed on concrete treated with  $9.8 \times 10^{-4}$  to  $1.9 \times 10^{-3}$  mg[AI]/per cm<sup>2</sup> hydroprene. In both tests, larvae were exposed and held at 27 or 32°C and 40, 57, or 75% RH. When larvae were exposed with no food to hydroprene for different time intervals, then transferred to untreated concrete containing flour, consistent effects were produced only at 144 h. At this exposure interval, the percentage of beetles arrested in the larval stage after 3–4 wk was generally greater at 75% RH compared with 40 and 57% RH, but there were no differences between species or temperature. The percentages of dead adult red flour beetles and live adults with morphological deformities were also greatest at 75% RH, and defects were more prevalent in red flour beetles than in confused flour beetles. When larvae were continually exposed to different concentrations of hydroprene on concrete that contained flour, the percentage of arrested larvae, dead adults, and live adults of both species generally increased with concentration. There were more deleterious effects at 75% RH compared with either 40 or 57% RH, and effects were more pronounced in the red flour beetle compared with the confused flour beetle. In both experiments, temperature effects were variable and inconclusive. Results indicate that continual exposure of last instar red flour beetle and confused flour beetle to hydroprene can limit population development, but exposure intervals of >6 d may be required for maximum effectiveness.

**KEY WORDS** beetles, hydroprene, treated surfaces, exposure, toxicity, morphological effects

THE INSECT GROWTH regulator (IGR) hydroprene has been registered for a number of years to control cockroaches in structures and dwellings. Adult emergence is often delayed or reduced when nymphs are exposed to hydroprene, and adults that do emerge are often deformed (King and Bennett 1988). Twisted and incomplete wings are common symptoms of exposure to hydroprene, and sterility is often associated with this and other morphological defects (King and Bennett 1989, Atkinson et al. 1992, Short and Edwards 1992, Reid and Bennett 1994). Actual and simulated field studies report successful eradication of cockroaches using either hydroprene alone or combined with reduced rates of conventional insecticides (Bennett et al. 1986, King and Bennett 1988, Edwards and Short 1993).

Several IGRs, including hydroprene, have been evaluated for efficacy toward stored-product beetle species (Oberlander et al. 1997). However, in most of these studies adults were exposed either on treated grain or diet or exposed by direct topical application, and toxicological effects were measured by inhibition of reproduction and larval development. Results have

been reported for a variety of species, including the red flour beetle, *Tribolium castaneum* (Herbst), and the confused flour beetle, *Tribolium confusum* Jacquelin du Val (McGregor and Kramer 1975, Loschiavo 1976, Amos et al. 1977, Hoppe 1981). Both species can infest stored grains, but they are more commonly found in food storage facilities, mills, and processing plants.

In recent years a new formulation of hydroprene (Gentrol, EPA registration No. 2724-351) has been registered to control stored-product insects in storage areas containing packaged or processed food. It is a 9.0% active ingredient concentrate, and it can be used as an aerosol spray or as a residual surface treatment. Label directions for surface application specify 28.6 ml of product in 3.8 liters to cover 141 m<sup>2</sup> (1 oz in 1 gallon for 1,500 feet<sup>2</sup>). There are no published reports of studies in which *Tribolium* spp. larvae have been exposed on treated surfaces to mimic field applications in warehousing facilities, nor are there studies that report the effects of concentration or exposure conditions on product efficacy. Also, most tests were conducted by exposing eggs in treated diet or on treated grain, and there are no reports where larvae were exposed on treated surfaces. The objectives of this study were to determine the effect of exposure interval on the response of last-instar red flour beetle and last-instar confused flour beetle larvae exposed to the

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label rate of hydroprene, the response of larvae exposed to different concentrations of hydroprene, the effects of temperature and relative humidity on efficacy of hydroprene, and the relative susceptibility of the two beetle species.

### Materials and Methods

**Experiment 1: Effects of Exposure Interval, Temperature, and Relative Humidity on Adult Emergence.** Twenty-five individual concrete treatment arenas were constructed in standard 100 by 15-mm petri dishes by mixing ready-mix concrete with water to create a liquid slurry (Arthur 1998), and filling each dish to an approximate height of 5 mm. Actual measured area of these concrete arenas was 62 cm<sup>2</sup>. After the concrete was poured the dishes were allowed to dry for 4 d on an open counter in the laboratory, where ambient temperature was  $\approx 25^{\circ}\text{C}$ . Humidity chambers were created by pouring 750 ml of saturated K<sub>2</sub>CO<sub>3</sub>, NaBr, or NaCl into the bottoms of each of three sets of plastic 26 by 36.5 by 15-cm boxes lined with a waffle-type grid (Arthur 1998). These three salt solutions maintained relative humidities of 40, 57, or 75%, respectively (Greenspan 1977).

The hydroprene formulation used in this test was the 9.0% (AI) ( $\approx 90$  mg [AI]/ml) emulsifiable concentrate (Gentrol). The label rate is 29.6 ml (1 fl. oz.) or 2.67 g in 3.784 liters (1 gallon) of water to cover 141 m<sup>2</sup> (1,500 feet<sup>2</sup>), therefore the equivalent amount needed for the area of the concrete arena was 0.0013 ml of Gentrol in a spray volume of 0.17 ml water ( $0.0013 \text{ ml} \times 90 \text{ mg/ml} = 0.117 \text{ mg [AI]}/62 \text{ cm}^2$  or  $1.9 \times 10^{-3} \text{ mg [AI]}/\text{cm}^2$ ). Four replicate solutions were formulated by mixing 0.38 ml of Gentrol in 50 ml of tap water, and a Badger 100 artists airbrush was used to spray each of four dishes in each replicate with aliquots of 0.17 ml. Confused flour beetle larvae were obtained from pesticide-susceptible colonies reared at 27°C and 60% RH. At these conditions pupation normally occurs five wks after eggs are laid and adults emerge a wk later. Ten 4-wk-old larvae, which were in the final instar, were exposed on each of 20 treated dishes and five untreated dishes. The treated dishes and larvae were put in one of the humidity chambers containing K<sub>2</sub>CO<sub>3</sub> and the five untreated controls were put in the other chamber. All boxes were put in an incubator set at 27°C, and temperatures and relative humidity within the boxes were monitored with HOBO recording sensors (Onset Computers, Pocasset, MA).

Larvae were exposed on the treated surfaces for 24, 48, 72, or 144 h at 27°C. Upon completion of the exposure interval, the treated and untreated dishes were removed from the humidity chamber and the incubator, and larvae were transferred to new petri dishes lined with filter paper and containing 500 mg of flour. These new dishes were put back into the respective humidity chamber, and held for an additional 3-4 wk to record adult emergence. At the conclusion of the test, the percentage of beetles that remained in the larval and pupal stages and the number of emerged

adults were recorded. Adults were classified as live or dead (having died either during the process of emergence or within a week of emergence), and also classified as being undeformed, having deformed wings, or grossly deformed. Adults determined to be grossly deformed had missing and deformed body parts and unsclerotized patches on the exoskeleton of the thorax and abdomen in addition to wing deformities.

Concrete arenas were constructed and testing procedures were repeated on successive weeks by exposing larvae in chambers containing either NaBr (57% RH) or NaCl (75% RH). After the series was completed with confused flour beetles at 27°C, testing procedures were repeated in exactly the same manner by exposing last instar red flour beetle larvae on treated concrete for the same time intervals at each of the three relative humidities. Once testing was completed at 27°C, a second set of studies was done with confused flour beetles and red flour beetles at each humidity at 32°C. New salt solutions were made and all testing procedures were exactly as described above.

The test was analyzed with species, temperature, relative humidity, and exposure interval as main effects and temperature  $\times$  relative humidity, relative humidity  $\times$  exposure interval, and temperature  $\times$  exposure interval as the interactions of interest, using the analysis of variance (ANOVA) Procedure (SAS Institute 1987). Response variables were the percentage of individuals that remained in the larval and pupal stages 5 wk after the larvae were exposed on the treated concrete, the percentage of adults that died within a week after emergence, the percentage of living emerged adults with deformed wings only, and the living emerged adults that were considered to be grossly deformed. All larvae on untreated concrete pupated and emerged as adults within 2-3 wk after the larvae were put on the arenas, no adults were deformed, and emergence was virtually 100% at all conditions. No corrections for mortality were necessary.

**Experiment 2. Effect of Concentration on Red Flour Beetle Larvae and Confused Flour Beetle Larvae Continuously Exposed to Hydroprene.** A second test was initiated at the same temperature and humidity conditions, but in this test different application rates were tested to simulate residual degradation. Two sets of three new humidity chambers containing saturated K<sub>2</sub>CO<sub>3</sub>, NaBr, or NaCl were created as previously described. The first series of tests was conducted at 27°C and 40% RH. Forty concrete arenas were constructed and allowed to dry and after the concrete dried, solutions were formulated by mixing 0.1, 0.2, 0.3, or 0.38 ml of Gentrol in 50 ml tap water. Application of these solutions at 0.17 ml/62 cm<sup>2</sup> gave concentrations of  $4.9 \times 10^{-4}$ ,  $9.8 \times 10^{-4}$ ,  $1.5 \times 10^{-3}$ , and  $1.9 \times 10^{-3}$  mg(AI)/per cm<sup>2</sup>, respectively.

After each concentration was sprayed on eight dishes,  $\approx 500$  mg of flour were put on each treated plus the two untreated controls for that concentration. For each concentration, 20 last instar red flour beetle larvae were put on each of four treated dishes and an untreated control and 20 last instar confused flour beetle larvae were put on each of the remaining four

**Table 1.** Percentage (mean  $\pm$  SE) of red flour beetles and confused flour beetles that failed to emerge from the larval stage after exposure of 4-wk-old last instars on concrete treated with hydroprene

Species	% RH	Exposure interval, h			
		24	48	72	144
Red flour beetle	40	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b
	57	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0b	5.0 $\pm$ 1.9ab	8.7 $\pm$ 2.9a
	75	0.0 $\pm$ 0.0a	7.5 $\pm$ 4.1a	10.0 $\pm$ 7.3a	6.2 $\pm$ 2.6ab
Confused flour beetle	40	0.0 $\pm$ 0.0a	1.2 $\pm$ 1.2a	0.0 $\pm$ 0.0b	5.0 $\pm$ 2.6a
	57	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	1.2 $\pm$ 1.2b	5.0 $\pm$ 2.6a
	75	2.5 $\pm$ 2.5a	3.7 $\pm$ 1.8a	8.7 $\pm$ 4.0a	6.2 $\pm$ 2.6a

For each species, means within columns followed by the same letter are not significant ( $P \geq 0.05$ , Waller-Duncan  $k$ -ratio  $t$ -test). Relative humidity ( $F = 10.2$ ;  $df = 2, 144$ ), exposure interval ( $F = 5.8$ ;  $df = 3, 144$ ), and temperature  $\times$  RH ( $F = 14.0$ ;  $df = 2, 144$ ) were significant ( $P \leq 0.01$ ). Temperature ( $F = 3.1$ ;  $df = 1, 144$ ), species ( $F = 0.1$ ;  $df = 1, 144$ ), relative humidity (RH)  $\times$  exposure interval ( $F = 2.1$ ;  $df = 6, 144$ ), and temperature  $\times$  exposure interval ( $F = 2.5$ ;  $df = 3, 144$ ) were not significant ( $P \geq 0.05$ ).

treated dishes and the untreated control. The treated dishes containing both species were stacked in one of the boxes containing  $K_2CO_3$  and the untreated controls were put in the other box. Both boxes were then put in an incubator set at 27°C and beginning two weeks after treatment, the dishes were checked weekly for 3 wk.

Testing procedures were replicated for studies at 27°C; 57 and 75% RH and for 32°C; 40, 57, and 75% RH. At each of the weekly assessments, the number of larvae, pupae, and emerged adults was recorded. Adults were classified as live or dead, and live adults were separated on the basis of morphological deformities as described for experiment 1. The test was analyzed with species, temperature, relative humidity, and concentration as main effects and temperature  $\times$  relative humidity, relative humidity  $\times$  concentration, and temperature  $\times$  exposure interval as the interactions of interest, using the ANOVA procedure (SAS Institute 1987). Response variables were the percentage of individuals that remained in the larval and pupal stages, the percentage of adults that died within a week after emergence, the percentage of living emerged adults with deformed wings only, and the living emerged adults that were considered to be grossly deformed. As in experiment 1, control emergence was usually 100% at all conditions, and no corrections for mortality were necessary.

## Results

**Experiment 1: Timed Exposure Study.** The percentage of red flour beetles and confused flour beetles

remaining in the larval stage after exposure on concrete treated with hydroprene was significant for relative humidity (RH) and exposure interval but not for species or temperature (Table 1). The temperature  $\times$  RH interaction was significant but neither the RH  $\times$  exposure interval nor the temperature  $\times$  exposure interval interactions were significant (Table 1). Data for each species were analyzed for differences between relative humidity at each exposure interval. When red flour beetle larvae were exposed for 48 and 72 h, more remained in the larval stage when exposed and held at 75 compared with 40% RH. Similar results occurred when confused flour beetle larvae were exposed for 72 h, but there was no difference among relative humidity at the other three exposure intervals.

The percentage of beetles remaining in the pupal stage was significant for species, temperature, relative humidity, exposure interval, and the temperature  $\times$  exposure interval interaction (Table 2). Although the overall ANOVA showed a difference among relative humidities, there were no significance differences ( $P \geq 0.05$ ) when relative humidity was analyzed by species, temperature, and exposure interval, so data for relative humidity were combined. Species and temperature were significant ( $P < 0.05$ ) only for larvae exposed for 144 h (Table 2). Fewer red flour beetles and confused flour beetles remained in the pupal stage when larvae were exposed and held at 32 compared with 27°C. When larvae were exposed for 144 h at 27°C, more red flour beetles than confused flour beetles remained in the pupal stage, but there was no difference between species at 32°C. Also, when larvae were exposed for 24, 48, or 72 h, nearly all of the

**Table 2.** Percentage (mean  $\pm$  SE) of red flour beetles and confused flour beetles that failed to emerge from the pupal stage after exposure of 4-wk-old last instars on concrete treated with hydroprene

Species	Temp, °C	Exposure interval, h			
		24	48	72	144
Red flour beetle	27	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	3.3 $\pm$ 2.2a	45.8 $\pm$ 6.8a
	32	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	2.5 $\pm$ 2.5b
Confused flour beetle	27	0.0 $\pm$ 0.0a	1.7 $\pm$ 1.2a	2.0 $\pm$ 1.9a	18.3 $\pm$ 5.2a
	32	0.0 $\pm$ 0.0a	0.8 $\pm$ 0.8a	2.5 $\pm$ 2.5a	1.7 $\pm$ 1.2b

For each species, means followed by the same letter are not significant ( $P \geq 0.05$ , PROC  $t$ -test). Species ( $F = 5.3$ ;  $df = 1, 144$ ) temperature ( $F = 50.0$ ;  $df = 1, 144$ ), relative humidity (RH,  $F = 3.8$ ;  $df = 2, 144$ ;  $P = 0.03$ ), exposure interval ( $F = 46.9$ ;  $df = 3, 144$ ), and temperature  $\times$  exposure interval ( $F = 37.8$ ;  $df = 3, 144$ ) were significant ( $P \leq 0.03$ ). Temperature  $\times$  RH ( $F = 1.7$ ;  $df = 2, 144$ ) and RH  $\times$  exposure interval ( $F = 1.8$ ;  $df = 6, 144$ ) were not significant ( $P \geq 0.05$ ). Data for relative humidity were not significant ( $P \geq 0.05$ ).

**Table 3.** Percentage (mean  $\pm$  SE) of adult red flour beetles and confused flour beetles that died within a week of emergence after exposure of 4-wk-old last instars on concrete treated with hydroprene

Species	Temp, °C	% RH	Exposure interval, h			
			24	48	72	144
Red flour beetle	27	40	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0a
		57	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	2.8 $\pm$ 2.8b	50.0 $\pm$ 28.9a
		75	0.0 $\pm$ 0.0a	5.3 $\pm$ 3.1a	16.6 $\pm$ 4.1a	25.9 $\pm$ 10.2a
	32	40	7.5 $\pm$ 7.5a	5.6 $\pm$ 3.3b	7.5 $\pm$ 4.8c	27.5 $\pm$ 11.9b
		57	8.3 $\pm$ 8.3a	7.8 $\pm$ 4.8b	41.3 $\pm$ 7.4b	35.8 $\pm$ 13.1b
		75	0.0 $\pm$ 0.0a	34.3 $\pm$ 11.4a	83.3 $\pm$ 5.6a	66.8 $\pm$ 10.6a
Confused flour beetle	27	40	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	2.5 $\pm$ 2.5a	46.4 $\pm$ 5.6a
		57	0.0 $\pm$ 0.0a	2.5 $\pm$ 2.5a	2.5 $\pm$ 2.5a	2.8 $\pm$ 2.8b
		75	2.5 $\pm$ 2.5a	3.1 $\pm$ 3.1a	9.4 $\pm$ 6.0a	4.2 $\pm$ 4.2a
	32	40	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 2.5b	0.0 $\pm$ 0.0b
		57	10.3 $\pm$ 7.1a	10.0 $\pm$ 7.1a	29.7 $\pm$ 13.8a	26.3 $\pm$ 6.6a
		75	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	11.1 $\pm$ 7.8a	30.9 $\pm$ 12.9a

For each species and temperature, means within columns for relative humidity (RH) followed by the same letter are not significantly different ( $P \geq 0.05$ , Waller-Duncan  $k$ -ratio  $t$ -test). Species ( $F = 21.5$ ;  $df = 1, 144$ ), temperature ( $F = 27.2$ ;  $df = 1, 144$ ), relative humidity ( $F = 10.0$ ;  $df = 2, 144$ ), exposure interval ( $F = 28.4$ ;  $df = 3, 144$ ), temperature  $\times$  RH ( $F = 9.4$ ;  $df = 2, 144$ ), RH  $\times$  exposure interval ( $F = 2.5$ ;  $df = 6, 144$ ), and temperature  $\times$  exposure interval ( $F = 4.5$ ;  $df = 3, 144$ ) were all significant ( $P \leq 0.03$ ).

beetles of both species emerged as adults if they were able to pupate, and the percentage of either species that remained in the pupal stage did not exceed  $3.3 \pm 2.2\%$ .

The percentage of adults that died within a week after they emerged was significant for species, temperature, relative humidity, exposure interval, and all three of the interactions (Table 3). The percentage of dead adults of either species was generally greatest at the 144-h exposures compared with the other three exposure intervals (Table 3). Dead adults were first analyzed by species, temperature, and exposure interval for differences among relative humidities. When red flour beetle larvae were exposed for 72 h at 27°C and for 48, 72, and 144 h at 32°C, there were more dead adults at 75 than at 40% RH ( $P < 0.05$ ). Similar results occurred when confused flour beetle larvae were exposed for 72 and 144 h at 32°C.

When data in Table 3 were analyzed by temperature, exposure interval, and relative humidity with respect to species, only five of the total of 24 possible comparisons were significant ( $P < 0.05$ ), and in four of these there were more dead red flour beetles than confused flour beetles. These were the 144-h expo-

sure at 27°C and 75% RH, and the 48, 72, and 144-h exposures at 32°C and 75% RH. The only time there were more dead confused flour beetles than red flour beetles was at the 144-h exposures at 27°C and 40% RH. Data were also analyzed by species, exposure interval, and relative humidity with respect to temperature. There were more dead adult red flour beetles at 32°C compared with 27°C at 57% RH, 48, 72, and 144-h exposures, and more dead adult confused flour beetles at 27 than at 32°C in larvae exposed for 144 h at 40% RH.

The percentage of live adults with wing deformities was significant for species, exposure interval, the temperature  $\times$  RH, and the RH  $\times$  exposure interval interactions (Table 4). Data for temperature were combined and analyzed by species and exposure interval for differences among relative humidities. At the 48 and 144 h exposures, there were more wing deformities in adult red flour beetles from larvae exposed at 75% RH compared with either 40 or 57% RH. There were no significant differences for confused flour beetle among relative humidity at any exposure interval. When data were compared by species, there were more red flour beetles with deformed wings than con-

**Table 4.** Percentage (mean  $\pm$  SE) of live emerged adult red flour beetles and confused flour beetles with wing deformities after exposure of 4-wk-old last instars on concrete treated with hydroprene

Species	% RH	Exposure interval, h			
		24	48	72	144
Red flour beetle	40	12.1 $\pm$ 7.4a	2.7 $\pm$ 1.7b	8.9 $\pm$ 4.0a	10.1 $\pm$ 4.4b
	57	22.0 $\pm$ 8.4a	2.5 $\pm$ 1.7b	13.2 $\pm$ 3.4	0.0 $\pm$ 0.0b
	75	10.0 $\pm$ 2.5a	29.2 $\pm$ 12.0a	12.3 $\pm$ 7.2a	62.5 $\pm$ 16.0a
Confused flour beetle	40	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	2.5 $\pm$ 2.5a	25.0 $\pm$ 16.4a
	57	0.0 $\pm$ 0.0a	3.0 $\pm$ 2.0a	3.6 $\pm$ 2.3a	2.1 $\pm$ 2.1a
	75	0.0 $\pm$ 0.0a	1.2 $\pm$ 1.2a	0.0 $\pm$ 0.0a	4.2 $\pm$ 4.2a

Means for relative humidity (RH) followed by the same letter are not significantly different ( $P \geq 0.05$ , Waller-Duncan  $k$ -ratio  $t$ -test). Species ( $F = 27.2$ ;  $df = 1, 144$ ), relative humidity ( $F = 6.1$ ;  $df = 2, 144$ ), exposure interval ( $F = 4.7$ ;  $df = 3, 144$ ), temperature  $\times$  RH ( $F = 5.6$ ;  $df = 2, 144$ ), and RH  $\times$  exposure interval ( $F = 3.5$ ;  $df = 6, 144$ ) were all significant ( $P \leq 0.04$ ). Temperature ( $F = 0.6$ ;  $df = 1, 144$ ) and temperature  $\times$  exposure interval ( $F = 2.1$ ;  $df = 3, 144$ ) were not significant ( $P \geq 0.05$ ). Data combined for temperature.

**Table 5.** Percentage (mean  $\pm$  SE) of red flour beetles and confused flour beetles that failed to emerge from the larval stage after exposure of 4-wk-old last instars on concrete treated with hydroprene

Species	% RH	Concentration of hydroprene (mg[AI]/cm <sup>2</sup> )			
		$4.9 \times 10^{-4}$	$9.8 \times 10^{-4}$	$1.5 \times 10^{-3}$	$1.9 \times 10^{-3}$
Red flour beetle	40	0.0 $\pm$ 0.0b	3.7 $\pm$ 1.9b	7.5 $\pm$ 4.8b	30.6 $\pm$ 13.9a
	57	0.0 $\pm$ 0.0b	2.5 $\pm$ 2.5b	10.6 $\pm$ 3.9ab	34.3 $\pm$ 7.2a
	75	7.5 $\pm$ 3.8a	23.7 $\pm$ 8.6a	26.2 $\pm$ 47.4a	22.5 $\pm$ 7.0a
Confused flour beetle	40	0.6 $\pm$ 0.6a	3.7 $\pm$ 1.6a	6.2 $\pm$ 2.1a	13.7 $\pm$ 3.7ab
	57	0.0 $\pm$ 0.0a	0.4 $\pm$ 0.4b	6.2 $\pm$ 3.3a	3.7 $\pm$ 1.2b
	75	4.4 $\pm$ 2.6a	4.4 $\pm$ 2.6a	16.2 $\pm$ 6.3a	20.8 $\pm$ 4.8a

For each species, means for relative humidity (RH) within each temperature followed by the same letter are not significantly different ( $P \geq 0.05$ , Waller-Duncan  $k$ -ratio  $t$ -test). Species ( $F = 23.9$ ;  $df = 1, 144$ ), RH ( $F = 11.9$ ;  $df = 2, 144$ ), concentration ( $F = 3.7$ ;  $df = 3, 144$ ), temperature  $\times$  RH ( $F = 28.8$ ;  $df = 2, 144$ ), and temperature  $\times$  concentration ( $F = 4.8$ ;  $df = 3, 144$ ) were significant ( $P \leq 0.02$ ). Temperature ( $F = 0.2$ ;  $df = 1, 144$ ) and RH  $\times$  concentration ( $F = 1.8$ ;  $df = 6, 144$ ) were not significant ( $P \geq 0.05$ ). Data combined for temperature.

fused flour beetle at the 24- and 72-h exposures at 57% RH, and at the 24, 48, and 144-h exposures at 75% RH. All adult red flour beetles and confused flour beetles classified as grossly deformed were dead, except for  $15.7 \pm 11.1\%$  live confused flour beetles in the 144-h exposures at 75% RH.

**Experiment 2: Continuous Exposure at Different Concentrations.** The ANOVA for the percentage of red flour beetles and confused flour beetles that remained in the larval stage after they were continually exposed to four rates of hydroprene was significant for species, relative humidity, and concentration but not for temperature (Table 5). The temperature  $\times$  RH interaction was significant, but neither the RH  $\times$  concentration nor the temperature  $\times$  concentration interactions were significant. Data for each species were analyzed for differences between relative humidity at each concentration. More red flour beetles remained in the larval stage when exposed and held at 75 than at 40% RH in all except the highest application rate. When confused flour beetles were exposed at the highest rate, there was no difference between 75 and 40% RH, and the lowest percentage of larvae occurred at 57% RH. There were no differences among relative

humidities for the other three concentrations. When data were analyzed by species, only the comparisons for  $9.8 \times 10^{-4}$  hydroprene, 75% RH, and  $1.9 \times 10^{-3}$  hydroprene, 57% RH were significant ( $P < 0.05$ ), and in both of these comparisons more red flour beetles than confused flour beetles remained in the larval stage. The percentage of beetles remaining in the pupal stage was not significant ( $P \geq 0.05$ ) for any main effect or interaction. Nearly all of the beetles that pupated eventually emerged as adults, with only  $0.2 \pm 0.07\%$  remaining in the pupal stage.

The percentage of adults that died within a week after emergence was significant for species, temperature, relative humidity, concentration, the temperature  $\times$  RH and the temperature  $\times$  concentration interactions (Table 6). At both 27 and 32°C, the percentage of dead emerged adults from red flour beetle larvae exposed on concrete treated with  $4.9 \times 10^{-4}$  and  $9.8 \times 10^{-4}$  ml/cm<sup>2</sup> hydroprene was generally greater at 75% than at either 40 or 57% RH. However, the percentage of dead adults seemed to fluctuate at the two lower humidities, and results were not consistent. There were no differences among relative humidities on concrete treated with  $1.5 \times 10^{-3}$  or  $1.9 \times$

**Table 6.** Percentage (mean  $\pm$  SE) of adult red flour beetles and confused flour beetles that died within a week of emergence after being exposed as 4-wk-old last instars on concrete treated with hydroprene

Species	Temp, °C	% RH	Concentration of hydroprene (mg[AI]/cm <sup>2</sup> )			
			$4.9 \times 10^{-4}$	$9.8 \times 10^{-4}$	$1.5 \times 10^{-3}$	$1.9 \times 10^{-3}$
Red flour beetle	27	40	10.4 $\pm$ 4.7b	97.5 $\pm$ 2.5a	100 $\pm$ 0.0a	100 $\pm$ 0.0a
		57	38.7 $\pm$ 9.9ab	72.1 $\pm$ 11.4b	96.1 $\pm$ 3.9a	100 $\pm$ 0.0a
		75	57.8 $\pm$ 12.7a	100 $\pm$ 0.0a	100 $\pm$ 0.0a	100 $\pm$ 0.0a
	32	40	6.2 $\pm$ 3.7b	76.2 $\pm$ 10.9ab	88.7 $\pm$ 11.2a	100 $\pm$ 0.0a
		57	0.0 $\pm$ 0.0b	55.0 $\pm$ 18.8b	87.9 $\pm$ 5.7a	100 $\pm$ 0.0a
		75	97.2 $\pm$ 2.8a	100 $\pm$ 0.0a	100 $\pm$ 0.0a	100 $\pm$ 0.0a
Confused flour beetle	27	40	0.0 $\pm$ 0.0a	10.9 $\pm$ 2.6a	41.8 $\pm$ 6.6a	70.4 $\pm$ 18.1b
		57	3.8 $\pm$ 1.3a	0.0 $\pm$ 0.0b	46.7 $\pm$ 12.4a	45.1 $\pm$ 15.6
		75	1.2 $\pm$ 1.2a	20.3 $\pm$ 6.1a	61.9 $\pm$ 13.9a	82.3 $\pm$ 7.5a
	32	40	1.2 $\pm$ 1.2b	6.4 $\pm$ 3.8b	11.6 $\pm$ 4.3b	30.7 $\pm$ 6.7b
		57	0.0 $\pm$ 0.0b	2.5 $\pm$ 2.5b	6.6 $\pm$ 5.1b	30.5 $\pm$ 11.6b
		75	20.5 $\pm$ 13.0a	51.5 $\pm$ 13.1a	69.3 $\pm$ 15.0a	91.7 $\pm$ 5.3a

For each species and temperature, means for relative humidity (RH) followed by the same letter are not significantly different ( $P \geq 0.05$ , Waller-Duncan  $k$ -ratio  $t$ -test). Species ( $F = 413.8$ ;  $df = 1, 144$ ), temperature ( $F = 4.1$ ;  $df = 1, 144$ ), relative humidity ( $F = 62.1$ ;  $df = 2, 144$ ), concentration ( $F = 117.3$ ;  $df = 3, 144$ ), temperature  $\times$  RH ( $F = 11.1$ ;  $df = 2, 144$ ), and temperature  $\times$  concentration ( $F = 2.9$ ;  $df = 3, 144$ ) were significant ( $P \leq 0.04$ ). RH  $\times$  concentration ( $F = 1.5$ ;  $df = 6, 144$ ) was not significant ( $P \geq 0.05$ ).

**Table 7.** Percentage (mean  $\pm$  SE) of live emerged adult red flour beetles and confused flour beetles with wing deformities after being exposed as 4-wk-old last instars on concrete treated with hydroprene

Species	% RH	Concentration of hydroprene (mg[AI]/cm <sup>2</sup> )			
		$4.9 \times 10^{-4}$	$9.8 \times 10^{-4}$	$1.5 \times 10^{-3}$	$1.9 \times 10^{-3}$
Red flour beetle	40	53.1 $\pm$ 9.9b	84.7 $\pm$ 6.7	—	—
	57	46.5 $\pm$ 6.6b	70.3 $\pm$ 11.3	93.7 $\pm$ 6.2	—
	75	94.7 $\pm$ 3.4a	—	—	—
Confused flour beetle	40	2.8 $\pm$ 2.2a	12.4 $\pm$ 2.6b	13.1 $\pm$ 4.4b	20.7 $\pm$ 2.2a
	57	10.6 $\pm$ 4.2a	8.2 $\pm$ 3.4b	8.2 $\pm$ 3.8b	17.1 $\pm$ 4.8a
	75	17.2 $\pm$ 7.1a	38.2 $\pm$ 8.8a	77.6 $\pm$ 7.7a	38.3 $\pm$ 13.6a

For each species, means within columns for relative humidity (RH) followed by the same letter are not significantly different (Waller-Duncan *k*-ratio *t*-test,  $P \geq 0.05$ ). Species ( $F = 250.3$ ;  $df = 1, 144$ ), RH ( $F = 16.9$ ;  $df = 2, 144$ ), concentration ( $F = 4.5$ ;  $df = 3, 144$ ), RH  $\times$  concentration ( $F = 5.5$ ;  $df = 6, 144$ ), and temperature  $\times$  concentration ( $F = 4.2$ ;  $df = 3, 144$ ) were significant ( $P < 0.01$ ). Temperature ( $F = 2.5$ ;  $df = 1, 144$ ) and temperature  $\times$  RH ( $F = 0.0$ ;  $df = 2, 144$ ) were not significant ( $P \geq 0.05$ ). Data combined for temperature. —, No live emerged adults at this condition.

$10^{-3}$  ml/cm<sup>2</sup> hydroprene, and the percentage of dead emerged adults ranged from  $87.9 \pm 5.7$  to 100%.

There were more dead emerged adult confused flour beetles at 75% than at either 40 or 57% RH at two of the application rates at 27°C and three of four rates at 32°C. (Table 6). The percentage of dead adults generally increased with application rate, but exceeded 80% on only two occasions. When data in Table 6 were analyzed for differences between species, there were only seven of 24 comparisons in which the percentage of dead emerged adults did not differ between the two species. These were 27°C: 40% RH,  $4.9 \times 10^{-4}$  ml hydroprene/cm<sup>2</sup> and 40 and 75% RH,  $1.9 \times 10^{-3}$  ml hydroprene/cm<sup>2</sup>; and 32°C: 40 and 57% RH,  $4.9 \times 10^{-4}$  ml hydroprene/cm<sup>2</sup> and 75% RH,  $4.9 \times 10^{-4}$  and  $1.5 \times 10^{-3}$  ml hydroprene/cm<sup>2</sup>. In all of the significant comparisons, there were more dead adult red flour beetles than confused flour beetles.

The percentage of live adults with wing deformities was significant for species, relative humidity, concentration, and the RH  $\times$  concentration and the temperature  $\times$  concentration interactions (Table 7). Data for temperature were combined and analyzed by species and exposure interval for differences among relative humidities. When larvae were exposed to the lowest concentration of hydroprene, there were more live red flour beetles with wing deformities at 75 than at 40 and 57% RH. No comparisons could be made among relative humidities for the other three concentrations because in five of eight conditions there were no live emerged adults. There were more live adult confused flour beetles with wing deformities at 75 than at 40 and 57% RH from larvae exposed on concrete treated with  $9.8 \times 10^{-4}$  and  $1.5 \times 10^{-3}$  ml hydroprene/cm<sup>2</sup>, but there were no differences among relative humidities at the other two concentrations.

All adult red flour beetles classified as grossly deformed were dead, except in the following treatments: 27°C, 57% RH,  $4.9 \times 10^{-4}$  and  $9.8 \times 10^{-4}$  ml hydroprene,  $1.5 \pm 1.5$  and  $21.1 \pm 10.6\%$  live, respectively. All adult confused flour beetles classified as grossly deformed were dead, except in the following treatments: 27°C, 40% RH,  $4.9 \times 10^{-4}$  and  $1.5 \times 10^{-3}$  ml hydroprene ( $2.5 \pm 2.5$  and  $8.5 \pm 5.5\%$  live, respectively), and 27°C, 57% RH,  $9.6 \times 10^{-4}$ ,  $1.5 \times 10^{-3}$ , and  $1.9 \times 10^{-3}$

ml hydroprene ( $7.5$ ,  $44.2 \pm 20.7$ , and  $50.9 \pm 17.4\%$  live, respectively).

## Discussion

Nearly all of the published research with IGRs and stored-product insects have involved exposures of either adults or eggs on treated grain or in treated diet, and efficacy was assessed by inhibition of progeny development (Oberlander et al. 1997). When eggs of stored-product beetles are exposed on grain treated with hydroprene, adult emergence is reduced and many of the emerged adults exhibit morphological abnormalities (Rup and Chopra 1984, Williams and Amos 1974). Morphological effects in adult cockroaches are also commonly associated with exposure of nymphs to IGRs (King and Bennett 1989, King and Bennett 1991, Atkinson et al. 1992, Short and Edwards 1992), but there is only one published study on the effects of IGRs on late instar *Tribolium* larvae (Hoppe 1981). Last instar red flour beetles were exposed on wheat flour treated with increasing concentrations of methoprene, and several effects were described depending on the concentration, including dead adults, live deformed adults with twisted elytron and/or wings, dead adults that failed to completely emerge from the pupa, and larvae that failed to pupate. Similar results were obtained in my studies when last instar red flour beetle and confused flour beetle larvae were exposed for different time intervals on concrete treated with hydroprene or on concrete treated with different concentrations of hydroprene. These last instars were susceptible to hydroprene when exposed on a treated surface, in a manner analogous to exposure in a field situation.

In published studies where cockroaches were exposed on treated surfaces, exposures were generally conducted at one set of temperature-humidity conditions (Atkinson et al. 1992, Short and Edwards 1992, Edwards and Short 1993). Published studies with IGRs applied to treated grain or treated diet also describe tests that were primarily conducted at one set of temperature-relative humidity conditions, usually within the range that is optimum for progeny production for the insect species used in bioassays. There are limited

data regarding the effects of temperature or humidity on the efficacy of IGRs. In one study by Samson et al. (1990), lesser grain borers, *Rhyzopertha dominica* (Fauvel), were exposed on rice treated with methoprene, fenoxycarb, or diflubenzuron. The rice was held at 25°C, 70 and 90% equilibrium relative humidity, and there was no consistent difference in efficacy of the IGRs due to humidity. No other reports document a temperature or humidity effect of hydroprene or other IGRs against stored-product insects exposed on raw grain. In both of my experiments, the percentages of arrested larvae, dead adults, and live adults with morphological deformities were often greater at 75% than at either 40 or 57% RH. Hydroprene does have volatile action when cockroaches are exposed on treated surfaces, and tends to bind more on porous surfaces than on smooth surfaces (Atkinson et al. 1992). One possible explanation for the increased activity against red flour beetles and confused flour beetles at 75% RH compared with 40 and 57% RH is that hydrolysis of the residues began to occur at the higher humidity, thereby releasing some of the residues on the concrete and increasing the activity of the hydroprene. Temperature effects in both experiments were variable and inconsistent.

When larvae were exposed on concrete treated with the label rate of hydroprene, then removed and placed on untreated concrete, exposure intervals of at least 144 h were required to consistently produce morphological and toxicological effects. However, when larvae were continuously exposed on concrete treated with increasing rates of hydroprene, the deleterious effects associated with hydroprene exposure were more consistent in both *Tribolium* species and increased as the concentration increased. Adult stored-product beetles are mobile and may be able to escape exposure on treated surfaces, but because larvae are not as mobile as adults, the long exposure interval required to produce consistent effects from hydroprene may not be a problem in field situations. Also, in these tests larvae were exposed with 500 mg of wheat flour, which was placed in the center of the exposure arena. The larvae distributed the flour over the entire surface, and the flour apparently absorbed some of the hydroprene residues, and the larvae came into contact with the residues from the surface and from movement through the flour. The presence of food material does not seem to have a negative effect on the efficacy of IGRs, and in the past many studies have been conducted in which IGRs are simply added to insect diet. In my experiments, the late instar larvae had to be provided with food or adult emergence was reduced in untreated controls, and extensive cannibalization occurred when adults began to emerge. However, survival of adult red flour beetles and confused flour beetles often increases when given food after exposure to contact insecticides or inert dusts (Arthur 1998, Arthur 2000).

Although temperature is controlled inside many indoor storage warehouses, relative humidity can fluctuate throughout the year, and is generally lower during the winter months. Large facilities may not be

climate-controlled, and temperature and relative humidity would be even more variable with the seasons. Efficacy of hydroprene toward the red flour beetle and confused flour beetle appears to be greater at 75% RH than at either 40 or 57% RH, but there was no serious loss in potency at the lower humidities. Also, last instar flour beetles appear to be slightly more susceptible to hydroprene than last instar confused flour beetles. This differential susceptibility between the two species should be taken into account when hydroprene is being used to control both species in storage facilities.

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### References Cited

- Amos, T. G., P. Williams, and R. L. Semple. 1977. Susceptibility of malathion-resistant strains of *Tribolium castaneum* and *T. confusum* to the insect growth regulators methoprene and hydroprene. *Entomol. Exp. Appl.* 22: 289–293.
- Arthur, F. H. 1998. Effect of a food source of red flour beetle (Coleoptera: Tenebrionidae) survival after exposure on concrete treated with cyfluthrin. *J. Econ. Entomol.* 91: 773–778.
- Arthur, F. H. 2000. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles: effects of temperature and relative humidity. *J. Econ. Entomol.* 93: 526–532.
- Atkinson, T. H., P. G. Koehler, and R. S. Patterson. 1992. Volatile effects of insect growth regulators against the German cockroach (Dictyoptera: Blattellidae). *J. Med. Entomol.* 29: 364–367.
- Bennett, G. W., J. W. Yonker, and E. S. Runstrom. 1986. Influence of hydroprene on German cockroach (Dictyoptera: Blattellidae) populations in public housing. *J. Econ. Entomol.* 79: 1032–1035.
- Edwards, J. P., and J. E. Short. 1993. Elimination of a population of the Oriental cockroach (Dictyoptera: Blattellidae) in a simulated domestic environment with the insect juvenile hormone analogue (S)-hydroprene. *J. Econ. Entomol.* 86: 436–443.
- Greenspan, L. 1977. Humidity fixed points of binary saturated aqueous solutions. *J. Res. Natl. Bur. Standards-Physics Chem.* 81A: 89–96.
- Hoppe, T. 1981. Testing of methoprene in resistant strains of *Tribolium castaneum* (Herbst) (Col., Tenebrionidae). *Z. Angew. Entomol.* 91: 241–251.
- King, J. E., and G. W. Bennett. 1988. Mortal. and developmental abnormalities induce by two juvenile hormone analogs on nymphal German cockroaches (Dictyoptera: Blattellidae). *J. Econ. Entomol.* 81: 225–227.
- King, J. E., and G. W. Bennett. 1989. Comparative activity of fenoxycarb and hydroprene in sterilizing the German cockroach (Dictyoptera: Blattellidae). *J. Econ. Entomol.* 82: 833–838.
- King, J. E., and G. W. Bennett. 1991. Sensitive developmental period of last-instar German cockroaches (Dictyoptera: Blattellidae) to fenoxycarb and hydroprene. *J. Med. Entomol.* 28: 514–517.

- Loschiavo, S. R. 1976. Effects of the synthetic insect growth regulators methoprene and hydroprene on survival, development or reproduction of six species of stored-product insects. *J. Econ. Entomol.* 69: 395–399.
- McGregor, H. E., and K. J. Kramer. 1975. Activity of insect growth regulators, hydroprene and methoprene, on wheat and corn against several stored-grain insects. *J. Econ. Entomol.* 68: 668–670.
- Oberlander, H., D. L. Silhacek, E. Shaaya, and I. Ishaaya. 1997. Current status and future perspectives of the use of insect growth regulators for the control of stored product insects. *J. Stored Prod. Res.* 33: 1–6.
- Reid, B. L., and G. W. Bennett. 1994. Hydroprene effects on the dynamics of laboratory populations of the German cockroach (Dictyoptera: Blattellidae). *J. Econ. Entomol.* 87: 1537–1546.
- Rup, P. J., and P. K. Chopra. 1984. Effect of hydroprene on *Callisobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. Stored Prod. Res.* 20: 229–232.
- Samson, P. R., R. J. Parker, and E. A. Hall. 1990. Efficacy of the insect growth regulators methoprene, fenoxycarb, and diflubenzuron against *Rhizopertha dominica* (F.) (Coleoptera: Bostrichidae) on maize and paddy rice. *J. Stored Prod. Res.* 26: 215–221.
- SAS Institute. 1987. SAS/STAT user's guide for personal computers, 6th ed. SAS Institute, Cary, NC.
- Short, J. E., and J. P. Edwards. 1992. Effects of hydroprene on development and reproduction in the Oriental cockroach, *Blatta orientalis*. *Med. Vet. Entomol.* 6: 244–250.
- Williams, P., and T. G. Amos. 1974. Some effects of synthetic juvenile insect hormones and hormone analogues on *Tribolium castaneum* (Herbst). *Aust. J. Zool.* 22: 147–153.

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